

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
Takumi TERATANI et al. : Group Art Unit: 1633
Serial No. 10/591,407 : Examiner: Quang NGUYEN
Filed: December 8, 2006 :
For: RAT EMBRYONIC STEM

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
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Sir:

I, Takahiro Ochiya, declare:

That I am a citizen of Japan, and my post office address
is National Cancer Center Research Institute, 5-1-1,
Tsukiji, Chuo-ku, Tokyo 104-0045, Japan;

That my education and employment history is as shown in my
Curriculum Vitae attached hereto (Attachment 1):

That I am a co-inventor of the above-identified U.S.
patent application SN 10/591,407 and directed and
supervised the following experiment, which was carried out
to determine the effect of rat leukemia inhibitory factor
(rLIF) on the efficiency of formation of rat inner cell
masses from rat blastocysts, the results of which follow
hereunder;

EXPERIMENTS

[Materials & Methods]

(1) Oocyte Sampling

A female WKY/N (Wistar Kyoto strain, Charles River
Laboratories Japan, 10-week-old or older) was naturally
crossbred, at 3 days after vaginal plug confirmation, the

female rat for oocyte sampling was sacrificed and the uterus was excised. The embryo was recovered and developed to become blastocyst (late stage) in 5% CO₂ incubator.

(2) Preparation of Feeder Cell

As a feeder cell to be used for the establishment and culture of rat ES cell, normal fibroblast of fetal mouse ICR at 12.5 days treated with mitomycin C was used (FIG. 2). Before use, a cryopreserved feeder cell was thawed one day before use, and cultured using STO medium (DMEM 450 ml, FBS 50 ml, Antibiotic-Antimicrototics solution 5 ml) and a gelatin-coated culture dish (Iwaki, Tokyo, Japan).

(3) Consideration of LIF Addition

The necessity of addition of a rat leukemia inhibitory factor (rLIF) to a culture medium during the step of formation of an inner cell mass from rat blastocyst was considered. Various concentrations (0-5000 units) of rLIF (Chemicon) were added during the step of formation of an inner cell mass from rat blastocyst, and the blastocyst was cultured for 7 days. The number of the inner cell masses formed was observed.

[Results]

As a result, inner cell masses were efficiently formed in the absence of LIF (30%), rather than in the presence of various concentrations of rLIF (0-16.7%) as shown below.

rat LIF (U)	No. of rat blastocysts	No. of rat inner cells masses formed
5000	6	0
2500	6	0
1000	6	1
500	6	0
100	6	1
0	10	3

That I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 11 day of November, 2009.



Takahiro OCHIYA

Curriculum Vitae

November 11, 2009

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1988 Osaka University, Graduate School of Medicine (Ph.D. awarded)

Professional History:

1988-1992 Research Assistant, Institute for Molecular and Cellular Biology, Osaka
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1993-1998 Section Head, Genetics Division, National Cancer Center Research Institute
1998- Section for Studies on Metastasis, National Cancer Center Research Institute
1991-1992 The Barnham Institute Medical Research, La Jolla, CA USA
2004- Visiting Professor, Waseda University, Graduate School of Science and
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2008- Visiting Professor, Tokyo Institute of Technology School and Graduate
School of Bioscience and Biotechnology

Society: Japan Society of Gene Therapy
Japanese Cancer Association
Japanese Biochemical Society
The Molecular biology Society of Japan

Publication

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